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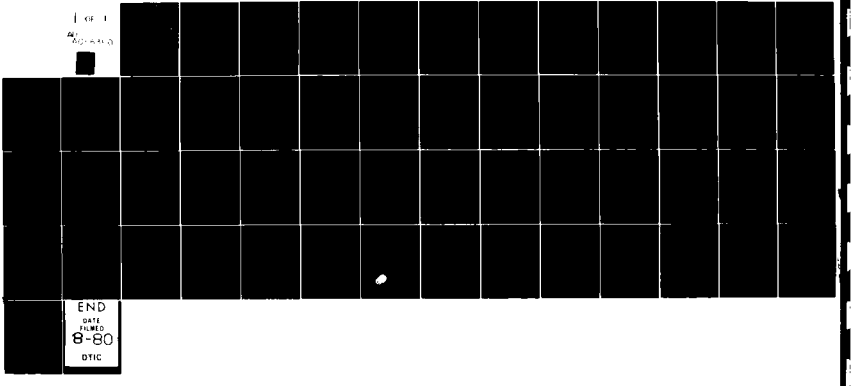

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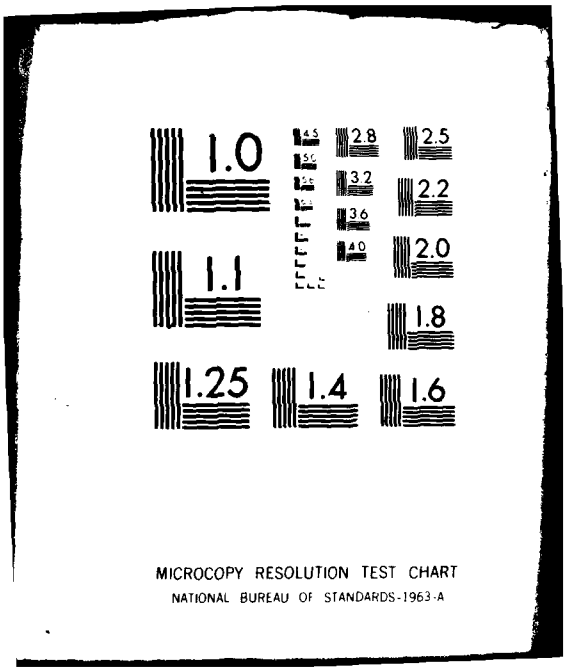
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CHEMOTHERAPY OF LEISHMANIASIS

Final Report

by

Wallace Peters, MD, DSc.

December 1978

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD17-77-G-9435

Liverpool School of Tropical Medicine
Pembroke Place, Liverpool L3 5QA
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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A086 350
	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) CHEMOTHERAPY OF LEISHMANIASIS	5. TYPE OF REPORT & PERIOD COVERED Final Jul 77 – Dec 78
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Wallace Peters M.D., D.Sc.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-77-G-9435
9. PERFORMING ORGANIZATION NAME AND ADDRESS Liverpool School of Tropical Medicine Pembroke Place Liverpool L3 5QA UK	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A 3M162770A802 00 075
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick Frederick, MD 21701	12. REPORT DATE Dec 78
	13. NUMBER OF PAGES 55
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

LEISHMANIA LEISHMANIASIS CHEMOTHERAPY ANTILEISHMANIAL

PENTOSTAM PENTOSTAM INDEX 8-AMINOQUINOLINES LEPIDINES

ANTIMONIAL LIPOSOMES

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Simplified screening procedures for in vivo evaluation in mice of potential antileishmanial compounds are described in this report. Data are provided on the antileishmanial activity of 12 WRAIR compounds and 6 other compounds against a visceral parasite, L.infantum (LV9), and two cutaneous parasites, L.major (LV39) and L.m.amazonensis (LV78).

The taxonomic status of a number of newly isolated strains of Leishmania is described and an updated list of isolates maintained in the reference

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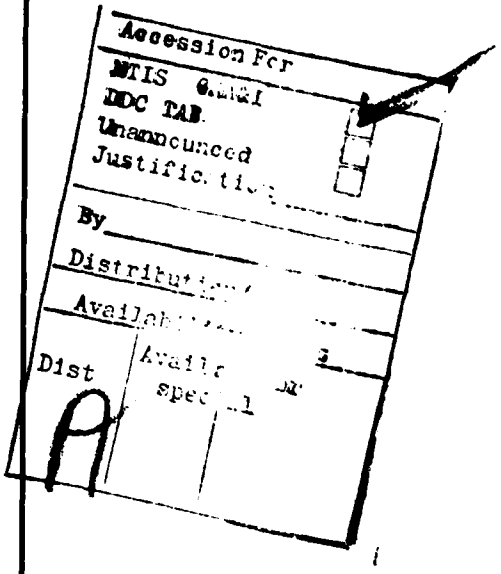
collection is appended. Other work has involved studies on antimonial leishmanicides in liposomes, separation of amastigotes from tissue using a cellulose column, purine and pyrimidine synthesis in L.m.amazonensis promastigotes and immunological studies of visceral and cutaneous leishmaniasis.



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INTRODUCTION

An interim Progress Report was submitted on the work of this unit in April 1978. This report included the test procedures now adopted for in vivo evaluation of potential antileishmanial activity. These are repeated below for convenience. A number of compounds was supplied by WRAIR for testing on four strains of Leishmania in December 1977. Preliminary data were supplied to WRAIR by the following month together with a request for additional supplies of certain drugs. Unfortunately, because of faulty communications we have only been in a position to continue with certain in vivo tests as from November 1978. The data requested by WRAIR a year ago are therefore not yet all available and top priority is currently being given to completing this work. Additional in vitro studies to parallel the in vivo work are also in hand at the present time.

Please note that the Progress Report sent in April 1978 was not intended for general circulation. It is replaced by the present Final Report which is now being submitted in the format recommended in a letter from WRAIR SGRD-AJ dated 11 October 1978.

SCIENTIFIC ACTIVITIES

1. CHEMOTHERAPY

1.1 Techniques

We have reviewed and simplified to some degree the procedures for drug testing that were set out in our application for research support, dated February 1977. Experience showed us that the original techniques developed by Mrs. Trotter, were unnecessarily cumbersome and did not give as consistent results as we had anticipated. The following techniques have now been adopted in this Department.

We are currently investigating the possible use of Balb/C mice as hosts to replace the NMRI line that we have used so far. Information received from colleagues in other centres suggests that Balb/C's may be as satisfactory as, or better than NMRI's and they are easier to obtain in bulk from breeders in this country than the latter variety.

1.1.1 Visceral leishmaniasis

The laboratory model used for the investigation of drug activity against visceral infection in this laboratory is L. donovani sensu lato (Strain LV9 from Ethiopia). The parasite is maintained by continuous passage in Syrian hamsters (Mesocricetus auratus). (This parasite is probably a variant of L. infantum).

Infected animals are anaethetised with ether and killed by cervical dislocation. The skin surface is swabbed with alcohol and the enlarged spleen removed aseptically into sterile Locke's solution in a sterile tissue homogeniser. After homogenisation the resultant suspension is centrifuged at 200 g for five minutes to remove tissue debris and the supernatant is then further centrifuged at 800 g for ten minutes to separate the amastigotes. These are resuspended in Locke's solution and their numbers counted in a Neubauer haemocytometer. The suspension is further diluted with Locke's solution to produce a final concentration of 50×10^6 amastigotes/ml.

20 g NMRI mice from a random bred colony are inoculated intravenously with 0.2 ml of the amastigote suspension. Parasites reach the liver and spleen rapidly by this route and may be detected one hour after infection. Infection is carried out randomly and the infected mice are subsequently divided at random into groups of six. Two untreated control groups are established and one treated group for each dose level of the compounds under test are set up. Additionally, Pentostam (sodium stibogluconate) at 400 mg base/kg is used as a positive drug control.

One control group is sacrificed on D+7 and liver impression smears are prepared, stained with Giemsa stain and examined to confirm the presence of parasites. Treatment of the dosed groups is commenced on D+8 and continued for five days. On the day following the final dose (D+13), all surviving animals are sacrificed and liver impression smears are prepared.

Assessment of the effect of treatment is made by examining liver impression smears. The smears are fixed with methanol, stained with filtered Giemsa stain and the numbers of parasites/1000 host cell nuclei are counted. The mean parasite count of each treated group is expressed as a percentage of the mean parasite count of the untreated control group and a dose response curve is plotted on log/probit graph paper to determine the ED_{50} and ED_{90} of the test compound.

The test result is also expressed, by comparison with Pentostam, as a Pentostam Index.

$$\frac{ED_{90}\ \text{Pentostam}}{ED_{90}\ \text{Test compound}} = \text{Pentostam Index}$$

1.1.2 Cutaneous leishmaniasis

Experimental compounds are tested against both Old World and New World forms of cutaneous leishmaniasis. The Old World type is represented by L. major = L. tropica major (Strain LV39 from USSR) and the New World cutaneous leishmaniasis by L. mexicana amazonensis (Strain LV78 from Brazil). The test system used is essentially the same for both species, differing only in the nature of the inoculum. L. major infections are produced by the intradermal inoculation of promastigotes from culture, whilst L. m. amazonensis infections are induced by the inoculation of amastigotes harvested from lesions in Syrian hamsters.

Studies are still proceeding to find a suitable rodent host for L. panamensis (Strain LIV641 = WR 128)

(i) Preparation of inocula

a) L. major

Using sterile techniques L. major amastigotes are isolated from developing nodules that have not ulcerated, by homogenisation and differential centrifugation. Rabbit blood agar slopes are seeded with a drop of the resultant amastigote suspension, 200 units/ml penicillin and 20 µg/ml streptomycin are added and the slopes are incubated at room temperature. Cultures are passed when the promastigotes are in their log phase of growth. Cultured promastigotes are concentrated by centrifugation at 800 g for ten minutes, resuspended in Locke's solution and the number of flagellates present estimated in a counting chamber. Suspensions are adjusted to contain between 5 and 25 x 10^6 promastigotes/ml.

b) L. mexicana amazonensis

A suspension of amastigotes is obtained by grinding tissue from infected hamster feet with sterile sand in Locke's solution using a pestle and mortar, and differentially centrifuging it at 200 g for five minutes and the supernatant at 800 g for fifteen minutes. The amastigote pellet is resuspended in Locke's solution, parasite numbers are counted in a haemocytometer, and the suspension is diluted to contain 5 x 10^7 amastigotes/ml.

(ii) Test procedure

20 g TFW mice are shaved over an area of 1.5 cm^2 above the tail base using animal clippers, and inoculated in random order with a volume of 0.2 ml of either L. major (or L. m. amazonensis) suspension intradermally 1 cm above the tail base in the midline. The animals are randomly caged in groups of five. The experiment is controlled with one untreated group and one positive drug control group which receives Pentostam equivalent to 400 mg antimony/kg. Four groups of mice, receiving a range of dose levels, are set up for each compound under test.

Treatment is commenced at three hours post-infection and continued daily for five days in all. Compounds are routinely administered subcutaneously in the interscapular region.

The site of infection is kept shaved throughout the experiment and the size and appearance of the lesion is assessed for each mouse weekly for up to seven weeks and a lesion score from 0-4 allocated as follows:

0 No thickening of skin
1 Thickening of skin
2 Small discreet nodule (up to 0.3 cm diameter) or extensive thickening
3 Discreet nodule (0.3-0.6 cm diameter)
4 Larger lesions and ulcerated lesions.

The lesion score is assessed by two workers each examining the animals independently and a mean lesion score is derived from the sum of both sets of readings in order to minimise the human error inherent in any such subjective system.

(iii) Determination of drug activity

a) Percentage suppression during the first four weeks

The sum of the weekly mean lesion scores for the first four weeks of the experiment in the treated groups is expressed as a percentage of the untreated control group score for the same period. These values are plotted on log/probit graph paper and the percentage suppression is determined.

b) Suppressive dose

The sum of the weekly mean lesion scores of the treated groups over the course of the whole experiment is expressed as a percentage of the untreated control values for the total period. The results obtained are plotted on log/probit graph paper and the SD_{50} and SD_{90} are determined. (SD_n = that dose which causes n% suppression of lesion development compared to the untreated control as reflected by the sum of the mean lesion scores.)

c) Pentostam Index

$$\frac{SD_{90}\ \text{Pentostam}}{SD_{90}\ \text{Test compound}} = \text{Pentostam Index}$$

1.2 Data on WRAIR and other compounds tested

Table 1 summarises data on 12 compounds provided by WRAIR and 6 other compounds. Details of the activity of these compounds as assessed so far against L. infantum (LV9), L. major (LV39) and L. mexicana amazonensis (LV78) in mice are provided in Tables 2 through 30. The outstanding points that arise from these data are the following:

(i) Glucantime sc is marginally less active than sodium stibogluconate (Pentostam) sc

(ii) Only 2 8-aminoquinolines showed significant activity against skin-dwelling parasites (namely WR 226292 and WR 226257). (Pentostam itself is only effective at high doses against these organisms.)

(iii) WR 226292 (the 5,6 methoxy analogue of WR 6026) is highly active against L. infantum and L. major when given po, (Pentostam Index 155) but little better than Pentostam given sc. WR 226292 is indeed the most active compound with the broadest spectrum of activity that we have encountered (other than Pentostam in liposomes versus L. infantum).

(iv) The lepidines WR 212579, and 226257 are less active than WR 6026 but WR 211666 is about as effective as WR 6026. All these compounds have a similar level of activity sc and po except WR 211666 which, like 226292 is more active po.

(v) WR 181023 (4-methyl primaquine) is about as active as WR 212579 against L. infantum but is ineffective at tolerated doses against the cutaneous species.

(vi) Clindamycin shows a modest activity against L. infantum.

(vii) Amphotericin B has poor activity against L. infantum but is superior to Pentostam against L. major.

(viii) An old batch of WR 6026 (AG 75499) showed poor activity compared to newer material. This may indicate chemical instability.

It is interesting to compare these data obtained in mice with data published recently by Kinnamon et al. (Am. J. trop. Med. Hyg., 1978, 27, 751) based on studies with the Khartoum strain of L. donovani (= L. infantum) in hamsters.

1.3 Studies on antimonial leishmanicides in liposomes

In association with the Department of Biochemistry in this University (Dr. New) we have been investigating the effects of incorporating Pentostam and other antimonials into liposomes, using L. infantum and L. major in NMRI mice as our target organisms. We were unaware that two other groups were working independently on this question, namely that of Black at the Nuffield Institute in London, and Steck at WRAIR. We have arrived at similar conclusions to both these groups, namely that the activity of organic antimonials is greatly enhanced against visceral leishmaniasis when the compounds are incorporated into liposomes. The activity against cutaneous infection is still being investigated, and information will be provided later. Other forms of liposome and other antileishmanial drugs will also be investigated in an attempt to enhance their therapeutic index and effectiveness. A report on our liposome studies is attached as Appendix I.

2. PARASITE BIOCHEMISTRY AND MODE OF ACTION

A new technique has now been described by Mr. Brazil for separating pure amastigotes from tissue using a cellulose column. (Appendix II). Preliminary data on the purine and pyrimidine synthetic activities of L. m. amazonensis promastigotes are summarised in Appendix III, details of which are being prepared for publication. This preliminary work shows a clear distinction between the metabolism of this parasite and others studied by Marr and Berens.

3. BIOCHEMICAL IDENTIFICATION OF LEISHMANIAL ISOLATES

This Department has now been officially designated as the "WHO International Collaborating Centre for the Biochemical Characterisation of Leishmania".

We have received several interesting new isolates including several from cases of Uta (from Dr. Herrer, Peru) and isolates from patients with kala-azar in the current Indian epidemic in Bihar. We have also received further material from Senegal isolated from man, rodents and P. duboscqui. This material is currently being grown and identified. The Indian material, all from man, is particularly interesting as it differs from strains of L. donovani that we received several years ago from Professor P. C. Sen Gupta of Calcutta.

A strain of Leishmania isolated by Mutinga and Ngoka from a dog in Kenya (Trans. R. Soc. trop. Med. Hyg., 1977, 71, 447) has proved to be identical to strains causing visceral disease in man in that area. This is the first time that the dog has been implicated as a reservoir of kala-azar in Kenya where the reservoir previously has been suspected to be rodents of various species.

We are currently examining all available isolates from kala-azar from India, the Middle East, Africa and South America in an attempt to resolve several enigmas that have arisen regarding the taxonomic status of the causative organisms. We still hope to receive more cultures of Honduras isolates from WRAIR to include in this survey.

The organisms responsible for cutaneous and mucocutaneous disease in the New World are currently receiving attention, and it is already becoming clear that mucocutaneous disease may result from infection with more than one species or subspecies of Leishmania.

A paper reviewing the biochemical taxonomy of isolates from the Aethiopian zoogeographical region has now been published (Appendix IV).

Further isolates have been received from WRAIR for identification and work on these is proceeding. The data available so far are as follows:

LV	Original designation			
639	117 Herrera	Man	Honduras	visceral
640	142	Animal	Honduras	visceral (trypanosome)
641	158 Murray	Man	Panama	cutaneous
642	182 Herrera	Man	Honduras	visceral
643	195	Animal	Honduras	visceral
644	220 Hendricks	Man		cutaneous
645	219	Man	India	visceral
646	234	Dog	Spain	cutaneous?
647				
648	LN158 Murray	Man	Panama	cutaneous
649	WR168 Khartoum	Man	Sudan	visceral
650	WR271	Man	Kenya	visceral
651	WR272 McGillivary	Man	Kenya	cutaneous
652	WR275 Orviss	Man		cutaneous

Preliminary characterisation of LV639, LV642 and LV644 indicate that the GPI and 6PGDH of all three are identical. These enzymes are quite different from other isolates from patients with visceral leishmaniasis of the Old World. Although the material has not yet been compared with markers for South American L. mexicana, the enzymes looked at so far appear to be very close to these organisms. Work is continuing on the identification of the whole series.

In Appendix V are listed isolates received in our WHO Collaborating Centre in addition to those listed as Annex 1 of our Final Technical Report of March 1977. The total number of isolates in our collection is now 677.

4. IMMUNOLOGICAL STUDIES

The ELISA test designed for the diagnosis of visceral leishmaniasis in our Department (Hommel et al., Ann. trop. Med. Parasit., 1978, 72, 213-218). has proved invaluable in identifying cases of kala-azar in India and helping to differentiate those probably suffering from malaria but misdiagnosed. This work is still continuing in collaboration with the staff of the National Institute of Communicable Diseases, Delhi, and will be reported in due course. Filter paper blood samples from man and a variety of domestic animals are being examined in Liverpool by Mrs. Sells, and those from man are being examined by the FAT by Dr. Lanotte of Montpellier University.

In vitro studies of T-cell function in cutaneous leishmaniasis and kala-azar are being conducted by Mrs. Sells. The leucocyte migration test is proving negative in patients known to have had Oriental Sore, but further investigations are being carried out including the use of modified techniques. A relationship between the type of cutaneous cellular response in infection with different Leishmania species is suspected to determine whether ELISA-detectable antibodies are produced in a given patient or not. This is being followed up in patients seen at clinics in England and overseas.

5. PUBLICATIONS

Papers published since last Final Report (March 1977)

Ashford, R. W. (1977). The comparative ecology of Leishmania aethiopica. Collogues Internationaux du CNRS, No. 239, 225-232.

Brazil, R. P. (1977). On the identity of Brazilian Leishmania strains and the use of the name L. braziliensis. Trans. R. Soc. trop. Med. Hyg., 71, 360-361 (correspondence).

Brazil, R. P. (1978). Electrophoretic variation of the enzyme phosphoglucomutase in different strains of Leishmania. Ann. trop. Med. Parasit., 72, 289-291.

Brazil, R. P. (1978). Isolation of the intracellular stages of Leishmania mexicana amazonensis using cellulose column. Ann. trop. Med. Parasit., 72, 579-580

Chance, M. L., Gardener, P. J. and Peters, W. (1977). The biochemical taxonomy of Leishmania as an ecological tool. Collogues Internationaux du CNRS, No. 239, 53-61.

Chance, M. L., Schnur, L. F., Thomas, S. C. and Peters, W. (1978). The biochemical and serological taxonomy of Leishmania from the Aethiopian zoogeographical region of Africa. Ann. trop. Med. Parasit., 72, 533-542.

Croft, S. L. (1977). Ultrastructural aspects of the nucleus of Leishmania hertigi. J. Protozool., 24, p. 42A (Abstract No. 14).

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Molyneux, D. H., Lewis, D. H. and Killick-Kendrick, R. (1977). Aspects of the microecology of Leishmania. Collogues Internationaux du CNRS, No. 239, 31-40.

New , R. R. C., Chance, M. L., Thomas, S. C. and Peters, W. (1978). The antileishmanial activity of antimonials entrapped in liposomes. Nature, 272, 55-56.

Peters, W., Chance, M. L., Mutinga, M. J., Ngoka, J. M. and Schnur, L. F. (1977). The identification of human and animal isolates of Leishmania from Kenya. Ann. trop. Med. Parasit., 71, 501-502.

Schnur, L. F. (1977). Insect flagellate excreted factors from leptomonad, crithidial and herpetomonad strains. J. Protozool., 24, p. 5A (Abstract No. 74).

Papers submitted for publication

Brazil, R. P. (1978). In vitro susceptibility of mouse peritoneal macrophages to Leishmania spp. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

Brazil, R. P. and McCarthy, J. D. (1979). Purine and pyrimidine synthesis in promastigotes of Leishmania mexicana amazonensis. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

Chance, M. L., New, R. R. C., Thomas, S. C. and Heath, S. (1979). The treatment of visceral leishmaniasis with liposomes. Trans. R. Soc. trop. Med. Hyg. (Laboratory Demonstration).

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Croft, S. L. and Schnur, L. F. (1979). The Noguchi-Adler phenomenon: an ultrastructural study of the effects of homologous antiserum on the growth of promastigotes of Leishmania braziliensis braziliensis and L. h. hertigi. Ann. trop. Med. Parasit.

Dedet, J.-P., Derouin, F., Hubert, B., Schnur, L. F. and Chance, M. L. (1979). Isolation of Leishmania major from Mastomys erythroleucus and Tatera gambiana in Senegal (West Africa). Ann. trop. Med. Parasit.

Rassam, M. B., Al-Mudhaffar, S. A. and Chance, M. L. (1978). Isoenzyme characterisation of Leishmania species from Iraq. Ann. trop. Med. Parasit.

RESEARCH PLANS

These were submitted to WRAIR in April 1978 with an application for renewal of WRAIR support. We have had no written information concerning the fate of this application but understand from a verbal communication that our application has been rejected. This is the subject of a letter to Dr. McCormick dated 20th November, 1978.

APPENDICES

Tables 1-30

Appendix I — Antileishmanial activity of antimonials entrapped in liposomes.
New,R.R.C.,Chance,M.L.,Thomas,S.C. and Peters,W. (1978),Nature,272,55-56.

Appendix II — Isolation of the intracellular stages of Leishmania mexicana amazonensis using cellulose column.
Brazil,R.P. (1978), Ann.Trop.Med.Parasitol.,72,579-580.

Appendix III — Purine and pyrimidine synthesis in promastigotes of Leishmania mexicana amazonensis.
Brazil,R.P. and McCarthy,J.D. (1979),
Trans.R.Soc.Trop.Med.Hyg.(in press)

Appendix IV — The biochemical and serological taxonomy of Leishmania from the Aethiopian zoogeographical region of Africa.
Chance,M.L.,Schnur,L.F.,Thomas,S.C.and Peters,W. (1978),
Ann.Trop.Med.Parasitol.,72,533-542.

Appendix V — Updated list of isolates in reference collection.

Table 1A

SUMMARY OF DATA FROM ANTILEISHMANIAL DRUG TESTS ON WRAIR AND OTHER COMPOUNDS.

WR	BN	LIV	*L.infantum* (=*L.donovani*) (LV9)		*L.major* (LV39)		*L.m.amazonensis* (LV78)		Comments
			ED_{90}	PI	SD_{90}	PI	SD_{90}	PI	
			155	1.0	2750	1.0	860	1.0	PENTOSTAM
6026 AD	BG 14463	1425	2.8	55.4	MTD		MTD		MTD = 30
6026 AD	BG 14463	1425	2.5p.o.	62.0					
6026 A1	AG 75499	1181	30	5.2	MTD		MTD		MTD = 30 ?Compound unstable(old batch)
226292 AA	BG 44541	1426	62	2.5	MTD				MTD = 30
226292 AA	BG 44541	1426	1.0p.o.	155	1.0p.o.	155	10p.o.	15.5	
211666 AB	BG 11417	1427	5.0	31.0	MTD		MTD		MTD = 30
211666 AB	BG 11417	1427	2.7p.o.	57.4					
211666 AB	BG 11417	1595	13.0	11.9					
212579 AB	BG 48969	1428	11.5	13.5	MTD				MTD = 30
212579 AB	BG 48969	1428	13.0p.o.	11.9					
226257 AA	BG 44425	1429	14.5	10.7	10	15.5			
226257 AA	BG 44425	1429	11.5p.o.	13.5					

ED_{90}/SD_{90} = mg/kg x 5 PI = Pentostam Index NA = not active MTD = maximum tolerated dose

(Note: all doses s.c. unless otherwise stated)

Table 1B

SUMMARY OF DATA FROM ANTILEISHMANIAL DRUG TESTS ON WRAIR AND OTHER COMPOUNDS.

WR	BN	LIV	*L.infantum* (=*L.donovani*) (LV9)		*L.major* (LV39)		*L.m.amazonensis* (LV78)		Comments
			ED_{90}	PI	SD_{90}	PI	SD_{90}	PI	
129577 AC	AW 41662	1430	145	1.1	100		100		
214975 AB	BE 45137	1593	200	0.8					GLUCANTIME
113254 AB	BB 48455	1187					100		
2975 E 19	AG 64745	1370	16.5	9.4	MTD		MTD		PRIMAQUINE PHOSPHATE MTD = 60
181023 AG	BE 50003	1373	9.8	15.8	60		100		4-METHYL PRIMAQUINE
181023	ZN 39806	1594	12.2	12.7					" "
182234	BE 17580	1596	270	0.6					2-METHYL PRIMAQUINE
203659 AC	BE 10429	1335	100	1.6					CLINDAMYCIN
		1349	30	5.2					U24729A
			950	0.2	1000	2.8			AMPHOTERICIN B
		1092	MTD		MTD				RIFAMPICIN MTD = 100
1544 BM	AR 20613	1488	MTD						CHLOROQUINE PHOSPHATE MTD = 100
1543	AR 78360	1495	MTD						MEPACRINE HYDROCHLORIDE MTD = 60

ED_{90}/SD_{90} = mg/kg x 5 PI = Pentostam Index NA = not active MTD = maximum tolerated dose

(Note: all doses s.c. unless otherwise stated)

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: Pentostam Route of administration: s.c.

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L23 Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	4	1610	
100.0 (as Sb) (30)	6	447	24.7± 7.1
200.0 (60)	6	132	7.3± 3.6
400.0 (120)	6	2	0.1± 0.1
600.0 (180)	6	0	0

ED_{50} 75(59-104) ED_{90} 155(123-215) Pentostam Index 1.0

as Sb 22.5(17.7-31.2) as Sb 46.5(36.9-64.5)

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.2	0.2	0.5	0.5	0.8	1.2	1.4			
600 (Sb) (180)	0.1	0.2	0.4	0.6	0.8	0.8	0.6	92.9	7.1	72.9
1000 (300)	0.2	0.3	0.4	0.4	0.7	0.6	0.8	92.9	7.1	70.8
1500 (450)	0.2	0.2	0.2	0.2	0.2	0	0.4	57.2	42.8	25.0
2000 (600)	0	0	0.2	0.2	0.2	0.2	0.2	28.6	71.4	20.8

SD_{50} 1050 SD_{90} 2750 Pentostam Index 1.0

as Sb 315 as Sb 825

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.6	1.0	1.3	1.3	1.4	1.4	2.3			
100 (as Sb) (30)	0.9	1.1	1.4	1.2	1.3	1.3	1.2	100	0	90.3
200 (60)	0.9	0.4	0.4	0.3	0.6	0.8	0.4	47.6	52.4	40.9
400 (120)	0.5	0.3	0.3	0.6	0.5	0.6	0.6	40.5	59.5	36.6
600 (180)	0.3	0.2	0.1	0.1	0.6	0.3	0.4	- 16.7	83.3	21.5

SD_{50} 290 SD_{90} 860 Pentostam Index 1.0

SD_{50} (as Sb) 87 SD_{90} as Sb 258

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1425 WR 6026AD BG 14463 Route of administration: SC

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals L6	L12	L41	Experiment No.: Mean amastigotes/1000 host cell nuclei L6	L12	L41	Date: % Control
Control	3	4	3	5460	4459	4050	
1.0			5			1360	33.6 ± 5.3
3.0	3			1357			24.9
10.0	3			103			1.9
30.0		6			0		0

ED_{50} 1.2 (0.6-1.9) ED_{90} 2.8 (1.5-4.6) Pentostam Index 55.4

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: L56 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.2	0.2	0.6	0.9	1.6	2.0	1.7			
3.0	0	0.3	0.4	1.4	1.6	2.0	2.4	100	0	100
10.0	0.5	0.8	1.9	1.0	2.0	2.8	2.9	100	0	100
30.0	0	0.1	0.4	1.0	2.0	2.2	2.7	32	68	100
60.0(~ LD80)	0	0	1.0	1.5	2.0	3.0	2.0	100	0	100

SD_{50} > MTD SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: L52 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.2	0.2	0.6	0.9	1.6	2.0	2.4			
3.0	0.6	0.5	0.7	1.3	1.2	1.8	2.7	100	0	100
10.0	0.2	0.3	0.7	0.4	0.5	1.6	1.6	94	6	87
30.0	0	0.4	0.4	1.0	0.9	2.0	2.0	53	47	100
60.0(~ LD80)	0	0	1.0	1.0	2.0	2.0	2.0	100	0	100

SD_{50} > MTD SD_{90} > MTD Pentostam Index -

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1413 WR 6026AD BG 14463 Route of administration: PO

L. donovani (Strain LV9) Experiment No.: L24 Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	1273	
1.0	5	514	22.6 ± 4.7
3.0	5	232	10.2 ± 4.2
10.0	5	28	1.2 ± 0.7

ED_{50} 0.4(0.2-0.6) ED_{90} 2.5(1.7-4.4) Pentostam Index 62.0

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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Table 3

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1141 WR 6026A-1 AG 75409 Route of administration: SC

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L43 Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	4600	
3.0	5	1490	32.4 ± 3.3
10.0	5	730	15.9 ± 3.1
30.0	5	560	12.2 ± 4.2
60.0(MTD)	5	125	2.7 ± 0.8

ED_{50} 2.2(1.0-3.9) ED_{90} 30(14-63) Pentostam Index 5.2

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: L47 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.1	0.4	0.6	0.6	1.3	1.9	2.4			
3.0	0.1	0.2	0.4	0.3	1.0	0.8	1.9	58.8	41.2	64.4
10.0	0.1	0.4	0.2	0.1	0.6	0.6	0.9	47.1	52.9	39.7
30.0	0.1	0.4	0.2	0.4	0.5	0.4	1.0	64.7	35.3	41.1

SD_{50} 7.0 SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: L48 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.4	0.5	0.8	0.9	1.2	0.7	1.0			
3.0	0.2	0.4	0.5	0.4	0.4	0.1	0.1	58	42	38
10.0	0.4	0.3	0.2	0.6	0.5	0.6	1.1	58	42	67
30.0	0.4	0.2	0.4	0.6	0.8	0.8	1.2	62	38	80
60.0 (LD80)	1.0	1.5	0.5	0.5	0.5	1.0	2.0	- 100	0	100

SD_{50} SD_{90} > MTD Pentostam Index -

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1426 WR 226292AA BG 44541 Route of administration: SC

L. donovani (Strain LV9) Experiment No.: L4 & L12 Date:

Dose (mg/kg)	No. of animals		Mean amastigotes/1000 host cell nuclei		% Control
	L4	L12	L4	L12	
Control	3	4	4525	4459	
30.0	3		4550		100
60.0	3		723		16.0
100.0		6		LD_{100}	-

ED_{50} ~ 50 ED_{90} ~ 62 Pentostam Index ~ 2.5

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
30.0	0	0	0	0.5	2.0	3.5	3.0	11.4	88.6	63.8
100.0	-	-	-	-	-	-	-	LD_{100}		

SD_{50} ~ 30 SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										
								-		

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/[illegible] WR 226292AA BG 44541 Route of administration: PO

L.donovani (Strain LV9) Experiment No.: L54 Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
1.0	5	28	1.2 ± 0.7
3.0	5	0	0
10.0	5	0	0

ED_{50} < 1.0 ED_{90} < 1.0 Pentostam Index > 155

L. major (Strain LV39) Experiment No.: L61 Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0.8	1.2	2.4	3.0	3.3	2.8	3.0			
1.0	0.4	0.2	0.1	0.2	0.2	0.2	0.6	12	88	12
3.0	0.4	0.4	0.2	0.4	0	0	0	19	81	8
10.0	0.4	0	0	0.4	0	0	0	11	89	5

SD_{50} < 1.0 SD_{90} ~ 1.0 Pentostam Index ~155

L. m. amazonensis (Strain LV78) Experiment No.: L53 Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	1.2	2.8	3.0	3.0	2.6	2.3	2.3			
1.0	0.6	0	0.2	0.4	0.8	0.7	0.8	12	88	20
3.0	0.2	0.2	0.8	0.6	0.7	1.0	0.9	18	82	26
10.0	0.4	0.2	0.4	0.6	0.5	1.0	1.2	16	84	25

SD_{50} < 1.0 SD_{90} > 10.0 Pentostam Index < 15.5

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 142 WR 211666AB BG 11417 Route of administration: SC

L. donovani (Strain LV9)	Experiment No.: L12 & L41		Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
	L12 L41	L12 L41	
Control	4 3	4459 4050	
1.0	5	2260	55.8 ± 11.6
3.0	5	1680	41.5 ± 5.9
10.0	6	198	4.5 ± 1.7
30.0	6	0	0

ED_{50} 1.9(0.8-3.2) ED_{90} 5.0(2.5-7.5) Pentostam Index 31.0

L. major (Strain LV39)	Experiment No.:									Date:
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
10.0	0	0	0.5	2.6	2.8	3.3	3.1	70.5	29.5	87.2
30.0	0	0	0	0.7	2.0	2.4	2.4	15.9	84.1	53.2

SD_{50} ~33 SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78)	Experiment No.:									Date:
Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.5	0.8	1.2	1.2	1.8			
10.0	0.1	0.5	0.8	1.2	1.4	2.2	2.1	100	0	100
30.0	0	0.2	0.7	1.0	1.0	1.8	2.0	100	0	100

SD_{50} > MTD SD_{90} > MTD Pentostam Index -

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV [illegible] WR-[illegible]LAB BG[illegible]

Route of administration: PO

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: [illegible] Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	2273	
1.0	5	1022	45.0 ± 8.4
3.0	5	394	17.3 ± 3.4
10.0	5	2	0.1 ± 0.1

ED_{50} 1.0(0.8-1.7) ED_{90} 2.7(1.9-4.2) Pentostam Index 57.4

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: [illegible] WR 2116[illegible]AB
BG 1141[illegible]

Route of administration: [illegible]

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: [illegible] Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	3120	
3.0	5	1470	47.2 ± 5.5
10.0	5	330	10.6 ± 1.2
30.0	5	130	4.2 ± 0.6

ED_{50} 2.4(1.6-3.5) ED_{90} 13(9.5-21) Pentostam Index 11.9

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV [illegible] WR [illegible]AB BG [illegible] Route of administration: SC

L.donovani (Strain LV9) Experiment No.: [illegible] Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	1030	
100	5(5 died)	-	
200	5(5 died)	-	
300	5(5 died)	-	

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1428 WR 212573AB BG 48969 Route of administration: SC

L.donovani (Strain LV9) Experiment No.: L12 & L18 Date:

Dose (mg/kg)	No. of animals L12	No. of animals L18	Mean amastigotes/1000 host cell nuclei L12	Mean amastigotes/1000 host cell nuclei L18	% Control
Control	4	4	4459	2515	
1.0		6		1920	76.4 ± 4.8
3.0		6		1632	64.9 ± 4.5
10.0	6		1890		42.4 ± 4.5
30.0(MTD)	6		0		0

ED_{50} 7.0(3.0-10) ED_{90} 11.5(5.4-17) Pentostam Index 13.5

L. major (Strain LV39) Experiment No.: L40 & L22 Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control L40	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
3.0	0.5	2.0	2.0	2.4	2.4	2.8	3.0	100	0	86.8
Control L22	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
10.0	0.3	0.7	0.8	1.3	2.2	3.3	2.9	70.5	29.5	81.6
30.0(MTD)	0.2	1.0	1.0	1.5	1.0	2.5	3.0	84.1	15.9	72.3

SD_{50} > MTD SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										
								-		

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1126 WR 212573AB BG 48969 Route of administration: P.O

L.donovani (Strain LV9) Experiment No.: 224 Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	2273	
3.0	5	1330	58.5 ± 4.2
10.0	5	668	29.4 ± 7.3
30.0	5	18	0.8 ± 0.5

ED_{50} 4.3(2.6-7.5) ED_{90} 13(8.0-23) Pentostam Index 11.9

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 14

Compound: LF /1429 WR 226257AA BG 44425 Route of administration: SC

L.donovani (Strain LV9) Experiment No.: L13 & L14 Date:

Dose (mg/kg)	No. of animals		Mean amastigotes/1000 host cell nuclei		% Control
	L13	L13	L13	L13	
Control	4	4	4020	2515	
1.0		6		1828	72.7 ± 8.1
3.0		6		1513	60.2 ± 5.0
10.0		6		1067	42.4 ± 2.9
30.0	6		95		2.4 ± 0.8
100.0	6		0		0

ED_{50} 5.3(3.2-9.5) ED_{90} 14.5(9.0-26) Pentostam Index 10.7

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
1.0	0.5	2.0	2.2	2.5	2.7	3.0	3.6	100	0	94.8
3.0	0.2	1.4	2.0	2.6	2.4	3.3	4.0	100	0	91.4
10.0	0.3	1.4	1.6	2.4	2.5	2.6	3.0	91.9	9.1	79.3

SD_{50} > 10.0 SD_{90} > 10.0 Pentostam Index -

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control										
								-		

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1429 WR 226257AA BC 14425 Route of administration: PO

L.donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	4050	
1.0	5	2570	63.5 ± 9.5
3.0	5	1760	43.5 ± 10.0
10.0	5	1590	39.3 ± 6.2

ED_{50} 3.6(1.0-8.0) ED_{90} 11.5(3.5-24) Pentostam Index 13.5

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 1c

Compound: LIV/1490 WR 183577AC AW 41661 Route of administration: SC

L.donovani (Strain LV9) Experiment No.: L45 Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	5	2070	
3.0	5	1710	82.6 ± 7.2
10.0	5	1390	67.2 ± 10.9
30.0	5	1100	53.1 ± 9.3
60.0	5	740	35.8 ± 3.3
100.0	5	363	17.5 ± 3.5

ED_{50} 20(9.5-44) ED_{90} 145(67-300) Pentostam Index 1.1

L. major (Strain LV39) Experiment No.: L50 Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0	0.1	0	0.8	0.8	1.4	1.6			
10.0	0	0	0	0.4	1.2	2.1	2.6	44	56	100
30.0	0	0.1	0	0.4	1.8	2.4	2.8	56	44	100
60.0	0	0.1	0	0.5	1.6	2.0	2.7	67	33	100
100.0	0	0	0	0.6	2.0	2.2	2.5	67	33	100

SD_{50} > 100 SD_{90} > 100 Pentostam Index -

L. m. amazonensis (Strain LV78) Experiment No.: L49 Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0.1	0	0.4	0.5	0.3	0.2	0.7			
10.0	0.1	0	0.4	0.2	0.7	1.0	1.3	70	30	100
30.0	0	0.1	0.4	0.3	0.5	0.4	0.5	80	20	95
60.0	0	0	0.4	0.2	0.3	0.5	1.5	60	40	100
100.0	0	0.4	0.4	0.4	0.3	0.7	1.2	100	0	100

SD_{50} > 100 SD_{90} > 100 Pentostam Index -

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1343 WR 214975AB
GLUCANTIME BE 45137

Route of administration: SC

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L65 Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	3120	
3.0	5	1950	62.6 ± 8.6
10.0	5	1910	61.3 ± 8.9
30.0	5	1630	52.3 ± 12.9

ED_{50} > 30 ED_{90} > 30 Pentostam Index -

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Table 15

Compound: LIV/1593 WR 214975AB
GLUCANTIME BE 45137

Route of administration: SC

L. donovani (Strain LV9) Experiment No.: Lv6

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	1030	
100	5	420	40.8 ± 19.2
200	5	170	16.5 ± 12.1
300	5	60	5.8 ± 1.9

ED_{50} 35 (12-90) ED_{90} 200 (70-500) Pentostam Index 0.8

L. major (Strain LV39) Experiment No.:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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Table 12

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1167 WR 113254AB BB 48455 Route of administration: SC

L.donovani (Strain LV9) Experiment No.: Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control			

ED_{50} ED_{90} Pentostam Index NOT TESTED.

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control	0.1	0.4	0.8	1.0	1.1	1.6	2.4			
3.0	0.1	0.6	1.3	0.5	1.1	1.6	1.2	100	0	86.5
10.0	0.1	0.4	0.6	0.6	1.6	2.1	2.2	73.9	26.1	100
30.0	0	0.1	0.4	0.5	0.9	1.1	1.6	43.5	56.5	62.6
100.0	0	0.4	0.4	0.5	1.1	1.6	1.2	56.5	43.5	70.3

SD_{50} > 100 SD_{90} > 100 Pentostam Index -

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TABLE 20

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1170 WR 2475E-19 AG 64745 PRIMAQUINE DIPHOSPHATE Route of administration: SC

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L9 & L41 Mean amastigotes/1000 host cell nuclei		Date: % Control
	L9 L41	L9	L41	
Control	3 3	5153	4050	
1.0	5		3040	75.1 ± 12.1
3.0	5		2390	59.0 ± 12.8
10.0	5		1000	24.7 ± 5.0
30.0	5	487		9.5 ± 1.6
60.0 (MTD)	5	10		0.2 ± 0.04

ED_{50} 4.6(2.7-9.0) ED_{90} 16.5(9.5-33) Pentostam Index 9.4

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: L40 & L22 Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control L40	0.4	1.0	2.0	2.8	3.8	3.6	3.8			
1.0	0.5	2.4	2.4	3.8	4.0	3.6	4.0	100	0	100
3.0	0.1	0.6	1.4	2.1	2.3	2.0	2.8	67.7	32.3	64.9
10.0	0.1	0.8	1.4	2.5	2.4	2.8	3.2	77.4	22.6	75.9
Control L22	0.1	0.4	1.3	2.6	2.8	3.6	3.3			
30.0	0	0	0.2	1.0	1.2	2.4	2.7	40.9	59.1	53.2
60.0 (MTD)	0	0	0	1.5	2.2	2.7	3.0	34.1	65.9	66.7

SD_{50} SD_{90} > MTD Pentostam Index -

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: L42 Weekly mean lesion score							Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
	1	2	3	4	5	6	7			
Control	0.1	0.4	0.5	0.8	1.2	1.2	1.8			
30.0	0	0.3	0.6	0.6	0.8	1.7	1.9	83.3	16.7	98.3
60.0 (MTD)	0	0	0.4	1.2	1.4	1.9	2.0	88.9	11.1	100
								-		

SD_{50} SD_{90} > MTD Pentostam Index -

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV/1373 WR 181023AG Route of administration: SC
4-METHYL PRIMAQUINE BE 50003

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L43 Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	4600	
3.0	5	1804	39.2 ± 9.8
10.0	5	450	9.8 ± 4.2
30.0	5	60	1.3 ± 0.6
60.0	5	10	0.2 ± 0.2
100.0	5	0	0

ED_{50} 2.7(1.6-4.5) ED_{90} 9.8(4.8-15.5) Pentostam Index 15.8

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.1	0.4	0.6	0.6	1.3	1.9	2.4			
3.0	0.1	0.2	0.4	0.7	0.5	0.4	1.2	82.4	17.6	50.7
10.0	0.1	0.2	0.6	0.5	0.7	0.8	1.0	82.4	17.6	53.4
30.0	0	0.2	0.4	0.4	0.8	0.8	1.2	58.8	41.2	52.1
60.0	0	0	0.3	0.5	0.7	1.3	1.3	47.1	52.9	56.2

SD_{50} > 60 SD_{90} > 60 Pentostam Index -

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: L48 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0.4	0.5	0.8	0.9	1.2	0.7	1.0			
10.0	1.0	1.0	0.5	0.6	0.6	0.7	1.1	100	0	100
30.0	0.4	0.5	0.6	0.6	1.0	0.7	1.0	73	27	87
60.0	0.4	0.6	0.7	0.4	1.1	0.7	1.3	81	19	95
100.0	0.6	0.6	0.6	0.6	0.6	0.8	1.3	92	8	93

SD_{50} > 100 SD_{90} > 100 Pentostam Index -

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Table ...

SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1394 WR 181023
4-METHYL PRIMAQUINE ZN 33604

Route of administration: SC

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	3	3120	
3.0	5	780	25.0 ± 6.2
10.0	5	460	14.8 ± 1.9
30.0	5	280	9.0 ± 1.2
100.0	5	0	0

ED_{50} 4.0(1.4-10.5) ED_{90} 12.2(4.3-32) Pentostam Index 12.7

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV [illegible] WR [illegible] Route of administration: [illegible]
8-METHYL PRIMAQUINE BE[illegible]

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: [illegible] Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	[illegible]	3110	
3.0	5	2510	[illegible]
10.0	5	2950	[illegible]
30.0	5	940	[illegible]

ED_{50} ED_{90} Pentostam Index

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: [illegible] Route of administration: [illegible]

L. donovani Strain LV9

Dose (mg/kg)	No. of animals	Experiment No.: [illegible] Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	[illegible]	1000	
100	[illegible]	250	24.[illegible] ± [illegible]
200	5(1 died)	213	20.0 ± 7.1
300	5(5 died)	-	-

ED_{50} 34(12-80) ED_{90} 270(90- MTD) Pentostam Index 0.6

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										
								-		

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

[illegible]

Compound: LIV [illegible] WR [illegible]AC
CLINDAMYCIN BE 104[illegible]

Route of administration: SC

L. donovani (Strain LV9)		Experiment No.: L[illegible]	Date:
Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	5460	
1.0	3	4710	86.[illegible]
3.0	3	3647	66.8
10.0	3	2090	38.3
30.0	3	1930	35.4
100.0	3	1633	29.9

ED_{50} 10 ED_{90} > 100 Pentostam Index < 1.6

L. major (Strain LV39)	Experiment No.:								Date:
Dose (mg/kg)	Weekly mean lesion score								
	1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78)	Experiment No.:									Date:
Dose (mg/kg)	Weekly mean lesion score									
	1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV [illegible] U24729A Route of administration: SC

L. donovani (Strain LV9) Experiment No.: [illegible] Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	3	5460	
30.0	3	2420	44.3

ED_{50} < 30 ED_{90} > 30 Pentostam Index < 5.2

L. major (Strain LV39) Experiment No.: Date.

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: AMPHOTERICIN B Route of administration:

L. donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	5	2[illegible]	
10.0	6	1517	[illegible] ± 3.9
30.0	6	898	34.9 ± 2.2
100.0	6	600	23.3 ± 3.4
300.0	6	527	20.5 ± 4.8

ED_{50} 11(4.5-32) ED_{90} 950(450- 1000) Pentostam Index 0.2

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0	0.9	1.5	2.1	2.9	3.2	3.2			
10.0	0	0.3	1.6	2.8	2.4	2.4	2.0	100	0	87
30.0	0	1.0	1.4	1.8	1.6	1.8	1.6	93	7	67
100.0	0	0	0.6	1.8	2.8	2.8	2.4	53	47	75
300.0	0	0.2	0.4	0.2	0.4	0.8	0.8	18	82	20

SD_{50} ~ 135 SD_{90} ~ 1000 Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV(2.5.)
RIFAMPICIN

Route of administration: P.O

L.donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: L35 Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	4	2575	
10.0	6	870	33.8 ± 6.3
30.0	6	809	31.4 ± 7.1
100.0	6	702	27.3 ± 6.9
300.0(LD_{50})	6	350	13.6
600.0(LD_{100})	6	--	-

ED_{50} 8.5(2.5-12.5) ED_{90} > MTD Pentostam Index -

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: L35 Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control	0	0.2	0.4	1.2	1.0	1.7	2.0			
60.0	0	0.2	0.4	1.0	1.2	1.2	1.4	89	11	83
100.0	0	0	0.4	1.0	1.3	1.3	2.5	78	22	100
300.0	0	0	0.2	0.8	1.6	1.6	1.6	56	44	89
600.0	0	0	0.2	0.6	0.7	1.0	1.2	50	50	78

SD_{50} SD_{90} > MTD Pentostam Index

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: LIV 1444 WR 1544BM
CHLOROQUINE PHOSPHATE AP 20413 Route of administration: S/

L. donovani (Strain LV9) Experiment No.: 223 Date:

Dose (mg/kg)	No. of animals	Mean amastigotes/1000 host cell nuclei	% Control
Control	4	1810	
60.0	6	1137	62.8 ± 15.6
100.0(MTD)	6	890	49.2 ± 3.6

ED_{50} ~ 100 ED_{90} > MTD Pentostam Index –

L. major (Strain LV39) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Experiment No.: Date:

Dose (mg/kg)	Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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SUMMARY OF ANTILEISHMANIAL DRUG TESTS

Compound: [illegible] WR 1543
METAKYNE HYDROCHLORIDE AR [illegible]

Route of administration: [illegible]

L donovani (Strain LV9) Dose (mg/kg)	No. of animals	Experiment No.: [illegible] Mean amastigotes/1000 host cell nuclei	Date: % Control
Control	4	1810	
30.0	6	1480	81.8 ± 10.7
60.0	6	827	45.7 ± 12.3

ED_{50} ~ 60 ED_{90} > 60 Pentostam Index -

L. major (Strain LV39) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

L. m. amazonensis (Strain LV78) Dose (mg/kg)	Experiment No.: Weekly mean lesion score 1	2	3	4	5	6	7	Sum Week 1-4 (as % control)	% suppression	Date: Sum Week 1-7 (as % control)
Control										

SD_{50} SD_{90} Pentostam Index NOT TESTED.

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(Reprinted from Nature, Vol. 272, No. 5648, pp. 55–56, March 2, 1978)

Antileishmanial activity of antimonials entrapped in liposomes

THE use of liposomes in chemotherapy as a method of ensuring that drugs reach the diseased or defective tissues suffers from the drawback that, on intravenous injection, the bulk of the material is rapidly cleared by the phagocytic cells of the liver[1], and does not reach the desired target. We report here a system in which advantage is taken of this behaviour to treat visceral leishmaniasis in mice, in which the amastigote parasites reside unharmed in vacuoles inside the Kupffer cells of the liver[2].

Liposomes were prepared from lecithin (purified from egg yolk by the method of Pangborn[3]) by ultrasonication as described by Bangham and Horne[4]. Lecithin (200 mg) was dispersed in 10 ml fluid, and sonicated in the cold for 5 min. For liposomes containing drug, 2 g either of antimony potassium tartrate or sodium antimony gluconate were included in the dispersion. Otherwise, the material was dispersed in distilled water alone. After sonication, the suspension was passed through a glass fibre filter pad, dialysed for 3 d against 2 l of normal saline (with three changes of medium) and then concentrated 10-fold using an Amicon XM100 filter. The quantity of antimonial drug retained in the concentrate was measured by atomic absorption spectroscopy. The filter held back up to 90% of the antimony contained in the dialysate. This material was found to sediment within 30 min at 100,000*g*. The above procedure gave liposomes containing typically a total of 5 mg drug. After storing for 1 week at room temperature, at least 50% of the antimony was retained within sedimentable liposomes, the remainder having escaped into solution. A fresh preparation was used for each experiment described. Figure 1 shows an example of an antimony-containing liposome which displays the characteristic multilamellar structure. Preliminary observation by X-ray microanalysis has provided additional evidence for the presence of antimony in these vehicles.

Fig. 1 Electron micrograph of section of mouse liver showing liposome containing potassium antimony tartrate 1 h after intravenous injection. Tissue fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer post-fixed in 1% osmium tetroxide and embedded in Araldite. (× 200,000).

Table 1 Survival of amastigotes in the liver after treatment with drug-loaded liposomes

Expt	Drug	Treatment	Dose administered (mg per kg body weight)	Parasites per 200 cell nuclei ± s.e.
1	Potassium antimony tartrate	None	None	240 ± 50
		Drug alone	20	260 ± 17
		*Drug within liposomes	20	0
2	Potassium antimony tartrate	None	None	849 ± 86
		Liposomes alone	None	863 ± 81
		Drug alone	20	760 ± 63
		†Drug + liposomes	20	304 ± 66
		*Drug within liposomes	20	0
3	Potassium antimony tartrate	None	None	297 ± 16
		*Drug within liposomes	0.125	247 ± 29
			0.25	225 ± 17
			0.5	199 ± 15
			1.0	113 ± 30
			2.0	101 ± 18
4	Potassium antimony tartrate	None	None	508 ± 86
		Drug alone	10	513 ± 34
		*Drug within liposomes	10	46 ± 19
			5	86 ± 21
	Sodium antimony gluconate	Drug alone	10	638 ± 22
		*Drug within liposomes	10	63 ± 15
			5	172 ± 29

Groups of six NMR1 mice were inoculated with amastigotes of *L. donovani* on day 0, and received their first dose of drug on day 10. In Expt 1, the drug was administered over 3 d (days 10, 11, 12) and the animals were killed on day 14. In Expts 2, 3 and 4, the drug was given as a single dose, and the livers taken for assay on day 12.
*Drug incorporated into liposomes during sonication. 1 mg of drug is contained in approximately 40 mg phospholipid.
†Drug mixed with a pure liposome suspension 10 min before injection.

The effect of drugs on a leishmanial infection was assayed by a modification of the method of Stauber *et al.*[5] using the mouse model of Bradley[6]. The concentration of drug was adjusted to 1 mg ml^{-1}, and injected intravenously into NMR1 mice (approximate weight 20 g) which had been inoculated intravenously 10 d previously with amastigotes of an Ethiopian isolate of *Leishmania donovani* s.l. (isolate no. HU3, Liverpool designation LV9). This parasite is maintained routinely by passage through hamsters and transferred to mice in the form of a spleen cell suspension. The mice were killed 2 d after injection of the drug, and the extent of the infection assayed microscopically by counting the number of amastigotes per 200 liver cell nuclei in an impression smear.

Table 1 shows that drug incorporated into liposomes had a much greater effect in reducing the parasite count in the liver than equivalent quantities of either liposomes or drug injected alone. Tartrate, 0.4 mg per animal (20 mg per kg body weight), given over a period of 3 d, was sufficient to abolish the infection completely as judged by light microscopy (Expt 1). An identical result was obtained when the same quantity was administered in a single dose (Expt 2). A significant reduction in the number of parasites was also observed after administration of a liposome suspension in which the tartrate had been dissolved at a concentration of 0.8 mg per ml just before injection. This result suggests that there may be some uptake of the drug by lecithin liposomes from the aqueous medium. Over the range of doses tested, there is a linear response of probit activity against log dose (Fig. 2). In addition, Table 1 shows that the enhanced activity exhibited by tartrate in the liposomes was also given by the drug

sodium antimony gluconate, which is formulated for clinical use under the trade name Pentostam (Burroughs Wellcome).

The ideal drug carrier has three important properties. First, it must protect the drug from most metabolic processes and, vice versa, protect the non-target host tissues during the time taken for the drug to reach the target. Second, it must transport the drug directly to the desired tissue, and, finally, it may enhance the uptake of the drug by the cells of the target tissue, and perhaps increase its efficacy within the defective cell.

In the experimental model described here, antimony-filled liposomes seem to satisfy most of these requirements. Antimony potassium tartrate was one of the first and most potent agents used for curing leishmaniasis,[1] but effective treatment necessitated prolonged exposure to highly toxic levels of drug. In our system, levels close to the lethal dose of 50 mg per kg were without effect; when entrapped in liposomes, however, a single dose of 20 mg per kg (Expt 2) was completely effective in eliminating the infection. Measurement of the concentration of antimony in plasma samples after injection of tartrate within liposomes showed that the drug was cleared from the blood with a half life of approximately 1 h. At this time, antimony-containing liposomes were found in the liver (Fig. 1). In contrast, the free drug was cleared rapidly from the plasma, and was not detectable by our method 15 min after intravenous administration. Lippincott *et al.*[7] have shown that free tartrate is taken up by erythrocytes during this time. Also, other workers have shown that liposomes are taken up by all organs encompassing the mononuclear phagocyte system (MPS)[8], and that uptake of therapeutic drugs into lysosomes of phagocytic cells can be enhanced *in vitro*[9] and *in vivo*[10] by incorporation of the drug into liposomes.

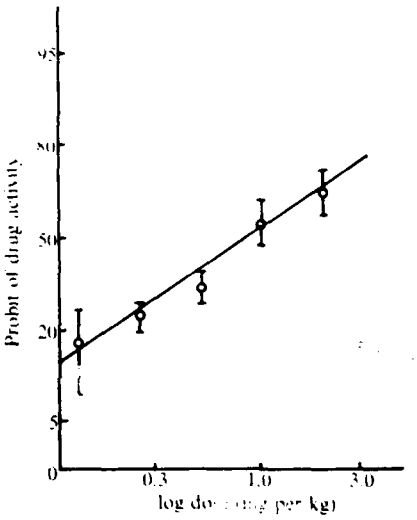

Fig. 2 Relation between probit of activity and dose of potassium antimony tartrate, using data from Table 1 (Expt 3).

In view of the fact that liposomes and leishmanial parasites share a propensity for cells of the MPS, it is reasonable to hope that targetting of antimonial drugs at present in use, by entrapment within liposomes, may be feasible in a range of clinical manifestations of the disease, and especially where amastigotes reside within macrophages of the liver, spleen, bone marrow and lymphatic system.

We thank Mr G. Moore for technical assistance, and Dr C. Green for helpful discussion. Financial support was provided by the Wellcome Trust, WHO and the US Army Research and Development Command (Contract DA-ERO-124-74-G0049).

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Received 20 October 1977; accepted 16 January 1978.

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Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset

Laboratory Demonstration presented at the Royal Society of Tropical Medicine and Hygiene Laboratory Meeting held in Liverpool, 16th November,1978.
Trans.R.Soc.Trop,Med.Hyg. 73(4)

PURINE AND PYRIMIDINE SYNTHESIS IN PROMASTIGOTES OF LEISHMANIA MEXICANA AMAZONENSIS.

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The occurrence of salvage pathway for purines is generally assumed in Leishmania. This assumption is made from the study of growth requirements of the organisms in defined medium (Steiger& Steiger,1977). There is little information on the uptake of radiolabelled nucleic acid precursors in Leishmania spp.(Croft,1977;Marr & Berens, 1978) and only recently have some of the enzymes of purine metabolism been examined (Konigk & Rasoul,1978).

In the present communication we investigated purines and pyrimidines metabolism in promastigotes of L.m.amazonensis using radiolabelled precursors. The incorporation of 2-^{3}H adenosine, 6-^{3}H thymidine, 5-^{3}H uridine, U-^{14}C glycine and 6-^{14}C orotic acid into nucleic acids was measured by scintillation counting methods(Neame et al..1974). Radioactive adenosine, thymidine, uridine and glycine were incorporated into the nucleic acids of the parasites, but there was no incorporation of orotic acid. These results show that promastigotes of L.m.amazonensis are capable of both salvage and de novo synthesis of purine, but they rely on the salvage pathway for pyrimidine synthesis.

The inability of L.donovani and L.braziliensis sensu lato to synthesize purines de novo(Marr & Berens 1978) could account for the different reaction of Leishmania spp. to different antileishmanial agents.

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APPENDIX V

Updated list
of isolates maintained in the reference collection

Key to donors

1	Dr. A. A. Herrer, Balboa Heights, Panama Canal Zone
2	Drs. R. Lainson and J. J. Shaw, Belem, Brazil
3	Professor W. H. R. Lumsden, London School of Hygiene and Tropical Medicine
4	Professor A. Zuckerman, WHO International Reference Centre for Leishmaniasis, Jerusalem, Israel
5	Hoffman La Roche, Basle, Switzerland
6	Professor F. Pifano, Caracas, Venezuela
7	Professor J. W. Torrealba, Valencia, Venezuela
8	Dr. J. Convit, Caracas, Venezuela
9	Professor D. Bradley, Ross Institute, London School of Hygiene and Tropical Medicine
10	Professor W. Barbosa, Goiania, Brazil
12	Colonel B. C. Walton, Balboa Heights, Panama Canal Zone
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14	Professor J. A. Rioux, Montpellier, France
15	Professor W. Mayrink, Belo Horizonte, Brazil
16	Liverpool School of Tropical Medicine
17	Professor J. Ranque, Marseille, France
18	Dr. R. Zeledon, San Jose, Costa Rica
19	Professor J. J. Laarman, Instituut voor Tropische Hygiene, Amsterdam
20	Professor R. Bray, Fajara, The Gambia
21	Professor V. Saf'janova, Gameleya Institute, Moscow
22	Dr. M. J. Mutinga, Nairobi, Kenya
23	Professor M. La Placa, Universita di Bologna, Italy
24	Dr. R. P. Brazil, Rio de Janeiro, Brazil
25	Dr. R. E. Abdalla, Khartoum, Sudan
26	Dr. M. Hommel, Liverpool School of Tropical Medicine
27	Dr. R. W. Ashford, Liverpool School of Tropical Medicine
28	Dr. Radim, University of Teheran, Iran
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31	Dr. N. Mansour, NAMRU-3, Cairo, Egypt
32	Dr. E. Tikasingh, Caribbean Epidemiology Centre, Trinidad
33	Dr. Grové, South African Institute of Medical Research, Johannesburg
34	Dr. A. Lemma, Addis Ababa, Ethiopia
35	Professor G. Piekarski, Institute of Medical Parasitology, Bonn, W. Germany
36	Dr. J. Baker, Molteno Institute, Cambridge
37	Professor P. C. C. Garnham, Imperial College Field Station, Ascot, England
38	Dr. P. Rees, University of Nairobi, Kenya
39	Captain L. Quigg, Brooke Army Medical Center, Texas, USA
40	Dr. J. Dedet, Institut Pasteur d'Algerie, Algeria.
41	Dr. A. Bryceson, Hospital for Tropical Diseases, London
42	Dr. D. Weinman, Yale University, New Haven, USA
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45	Dr. A Ebrahimzadeh, Jondi Shapur University, Iran
46	Dr. J. Golenser, Institute of Medical Parasitology, University of Nijmegen
47	Dr. F. Sukkar, Ministry of Health, Baghdad
48	Dr. F. Neva, National Institutes of Health, Bethesda, USA.

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50 Dr. D. Le Ray, Prince Leopold Institut de Medecine Tropicale, Antwerp, Belgium
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56 Dr. P. Desjeux, Institut Pasteur, Dakar, Senegal
57 Dr. R. Custodio, Tegucigalpa, Honduras
58 Dr. M. Reguer, Institut Pasteur, Cayenne, French Guyana

Key to abbreviations used in notes

A	amastigotes available
CL	cutaneous lesion
DCL	disseminated cutaneous leishmaniasis
KA	kala-azar
LR	leishmaniasis recidivans
LRC	leishmaniasis reference collection (Jerusalem)
MCL	multiple cutaneous lesions
MUC	mucocutaneous
PKDL	post kala-azar dermal leishmanoid
SCL	single cutaneous lesion
V	visceral
NS	normal skin
lump	London University Medical Parasitology
T	trypanosome

Appendix V. Updated list of isolates maintained in the reference collection.

LIV626	L.major	DK81	Dedet(40)	Senegal	Man	CL
LIV627	L.major	DK83	Dedet(40)	Senegal	Man	CL
LIV628	L.major	DK84	Dedet(40)	Senegal	Man	CL
LIV629	L.major	DK85	Dedet(40)	Senegal	Man	CL
LIV630	L.major	DK86	Dedet(40)	Senegal	Man	CL
LIV631	L.major	DK87	Dedet(40)	Senegal	Man	CL
LIV632	L.donovani	D1	Mutinga(22)	Kenya	Dog	
LIV633	L.donovani	D2	Mutinga(22)	Kenya	Dog	
LIV634	T.roussetti	-	Hommel(26)	Gabon	Russetus aegypticus	
LIV635	L.donovani	I	Chowdhury(51)	India	Man	KA
LIV636	L.donovani	II	Chowdhury(51)	India	Man	KA
LIV637	L.donovani	VI	Chowdhury(51)	India	Man	KA
LIV638	L.donovani	VIII	Chowdhury(51)	India	Man	KA
LIV639	L.sp.	Herrera 117	Hendricks(53)	Honduras	Man	KA
LIV640	L.sp.	142	Hendricks(53)	Honduras	Animal	V
LIV641	L.sp.	Marray 158	Hendricks(53)	Panama	Man	CL
LIV642	L.sp.	Herrera 182	Hendricks(53)	Honduras	Man	KA
LIV643	L.sp.	195	Hendricks(53)	Honduras	Animal	V
LIV644	L.sp.	Hendricks 220	Hendricks(53)		Man	CL
LIV645	L.donovani	219	Hendricks(53)	India	Man	KA
LIV646	L.sp.	234	Hendricks(53)	Spain	Dog	skin
LIV647	L.sp.					
LIV648	L.b.panamensis	Murray LN158	Hendricks(53)	Panama	Man	CL
LIV649	L.donovani	Khartoum WR168	Hendricks(53)	Sudan	Man	KA
LIV650	L.donovani	WR271	Hendricks(53)	Kenya	Man	KA
LIV651	L.sp.	McGillivary WR272	Hendricks(53)	Kenya Nakuru	Man	CL
LIV652	L.sp.	Dwiss WR275	Hendricks(53)		Man	CL
LIV653	L.donovani	[illegible]/SKC		India	Man	KA
LIV654	L.braziliensis		Reguer(58)	French Guyana	Man	CL
LIV655						
LIV656						
LIV657	L.donovani	RB3	Behin(54)	Sudan	Man	KA
LIV658	L.tropica	D76	Abdalla(25)	Sudan	Man	CL
LIV659	L.mexicana		Brazil(24)	Brazil Maranhao	Man	DCL
LIV660						

Appendix V. Updated list of isolates maintained in the reference collection.(continued)

LIV661	L.sp.	Volpes V61	Bettini(55)	Italy,Grossetto	Fox	
LIV662	L.donovani	Lana	Bettini(55)	Italy,Orbitello	Dog	
LIV663	L.donovani	Dora	Bettini(55)	Italy,Grossetto	Dog	
LIV664	L.donovani	Cane	Bettini(55)	Italy,Puglie	Dog	
LIV665	L.sp.	DK110	Desjeux(56)	Senegal	Mastomys sp.	V
LIV666	L.sp.	DK115	Desjeux(56)	Senegal		
LIV667	L.sp.	L69	Rioux(14)	France	Man	KA
LIV668	L.sp.	L70	Rioux(14)	France	Man	KA
LIV669	L.sp.	L71	Rioux(14)	France	Man	KA
LIV670	L.sp.	L72	Rioux(14)	France	Dog	V
LIV671	L.sp.	L73	Rioux(14)	France	Man	KA
LIV672	L.sp.	L76	Rioux(14)	France	Man	KA
LIV673	L.sp.	L77	Rioux(14)	Tunisia	Dog	V
LIV674	L.sp.	L78	Rioux(14)	Tunisia	Dog	V
LIV675	L.donovani	B14	Rioux(14)	Senegal	Dog	V
LIV676	L.hertigi	CM170	Zeledon(18)	Costa Rica	Coendou mexicana	
LIV677	L.sp.	Salem	Custodio(57)	Honduras	Man	CL

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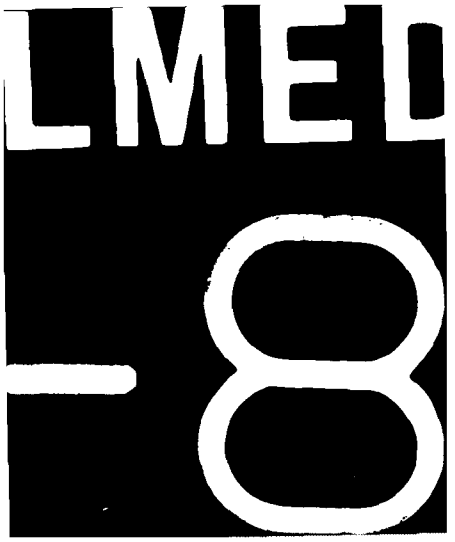
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